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May 4, 2012

Dr. Neil Carriker
Program Manager, Special Projects
Tennessee Valley Authority Kingston Ash Recovery Project
Kingston, Tennessee

Dr. Carriker,

Subject: Preliminary Evaluation of 2011 ORNL Results: *Fish Health*

We are pleased to submit to you our preliminary evaluation of 2011 *Fish Health* Task results.

The enclosed report covers the initial analysis of the fish health metrics collected from fish from the 2011 spring and fall fish collections.

We hope this preliminary update is useful to you. If you have any questions or require additional information, please call me at (865) 576-0266.

Sincerely,

Mark Bevelhimer, PhD
Principal Investigator, Fish Health
Environmental Sciences Division
Oak Ridge National Laboratory

c: M. J. Peterson
 D. Jones, ARCADIS

Preliminary Evaluation of 2011 ORNL Results: *Fish Health* **May 4, 2012**

Introduction

This report summarizes the results of samples collected in spring (April-May) and fall (October-November) 2011 for the fish health task for the Kingston Fossil Plant Coal Ash Spill Project. As in 2009 and 2010, fish were collected in 2011 from reference sites (ERM 8.0, LERM 2.0, CRM 8.0) and sites at and downstream of the Kingston fly ash spill (ERM 3.0, ERM 1.5, CRM 1.5). A variety of fish health measures were collected from 8-10 fish of each of four species from each site -- bluegill, largemouth bass, and redear sunfish in the spring and bluegill, largemouth bass, and channel catfish in the fall. The specific objective of the fish health component of the overall biological monitoring program is to determine if exposure to fly ash-associated metals causes short, intermediate, or long-term health effects on these sentinel fish species. The fish health study conducted in conjunction with the bioaccumulation and reproductive study is critical for assessing and evaluating possible causal relationships between contaminant exposure (bioaccumulation) and the response of fish to exposure as reflected by the various measurements of fish health.

Preliminary Results

The early analysis of the 2011 fish health data concentrated on comparing 2011 values to the data collected in 2009 and 2010. For a rapid assessment, we created data blocks of the mean values for each species-metric combination arranged by site and collection period (season/year) and then color-coded so that we could make easy visual analysis (Tables 1 and 2). (Note: not all metrics are included in these tables.) Values were color-coded such that the lowest values are the lightest color and highest values the darkest. Specifically, we looked for evidence of:

1. Higher or lower values at any of the three spill sites as compared to the three reference sites
2. A consistent increase or decrease at any of the spill sites from 2009-2011 (that was not also evident at the reference sites).
3. Decreasing or increasing trend within the spill sites in a downstream direction (i.e., ERM 3.0, ERM 0.9, CRM 1.5)

The visual assessment did not reveal any consistent patterns that suggest an obvious health effect of exposure to the fly ash spill; however, we did make the following observations of the 2011 data that will require further evaluation:

Bluegill

- Spring visceral somatic index was higher at all sites than in 2010 (that's a good thing)
- Spring blood protein was high at ERM 0.9 (a good thing)
- Spring blood sodium was highest at spill sites (a good thing)
- Spring stomach fullness was low at ERM 0.9
- Spring condition factor (CF) was lowest at two of the spill sites
- Spring liver-somatic index (LSI) was lowest at the spill sites just like in 2010
- Fall protein and calcium higher at ERM 0.9 than other sites (a good thing)
- Fall stomach fullness low at spill sites especially ERM 0.9

Largemouth Bass

- Spring hematocrit at spill sites lower than in 2010
- Spring glucose is higher at spill sites than reference sites and lower than in 2010
- Spring blood protein is slightly lower than in 2010
- Spring gall bladder color higher than 2010 with spill sites highest
- Spring CF highest at three spill sites (a good thing)
- Spring LSI lowest at ERM 0.9
- Fall BUN elevated at ERM 3.0
- Fall sodium slightly lower at most sites
- Fall CF slightly lower at spill sites

With small sample sizes (10 or less per species per site per period in this study) most statistically significant results should be apparent in a careful visual assessment of the data. We saw little here, but we caution that conclusions should not be made until the appropriate statistical tests are applied to these data.

Future Analysis

Future analyses will include a rigorous statistical analysis to determine if there are significant differences in the health metric values among reference and spill sites and from year to year. This analysis will include the multivariate canonical discriminant analysis that has been performed on past years' data.

We also intend to combine the fish health results and the bioaccumulation results in an analysis to look for any relationships between contaminant body burden and fish health response. Because both sets of data were collected from the same individual fish this should be a very powerful analysis and will overcome possible problems with highly mobile species, such as largemouth bass and catfish, whose spot of capture may not actually reflect the amount of exposure to fly ash that can be inferred by a less mobile species, such as bluegill.

In addition to the data presented here, select 2011 samples of gill, liver, and gonad tissue will soon be analyzed for histopathological anomalies as in previous years, and these results will be included in the multi-parameter analyses.

Table 1. Fish health metrics for 9 blood parameters for bluegill and largemouth bass collected at 6 sites over three years. Parameters include ALBumin, ALkaline Phosphatase, ALanine Transaminase, AMYlase, Blood PROtein, Blood Urea Nitrogen, CAlcium, CREATinine, and GLOBulin. Cells within each species group are color-coded with lowest values in light shades and highest values in dark shades.

Row Labels	ALB						ALP						ALT								
	2009		2010		2011		2009		2010		2011		2009		2010		2011				
	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall			
BLUGIL																					
Ref	CRM8.0	16.1	14.3	19.9	19.1	19.9	18.4	####	37.7	24	28.6	21.5	30.5	16.9	####	22.4	13.2	28.5	14.3	31.5	19.1
Ref	ERM8.0	15.6	16.4	22.3	18.9	21.8	21.1	####	30.8	28.4	36.4	22.9	52.4	8.33	####	21.5	17.1	20.1	13.3	25.2	12.2
Ref	LERM2.0		16.7	20	20	24	24.7	####		22.2	34.8	14.4	23	8.67	####		9.1	17.7	14.3	15.6	16.4
Spill	ERM3.0	15.6	13.9	19.9	19.8	23.1	21.4	####	27.5	24.3	36.5	15.5	23.3	3.33	####	20	13.1	20.5	19.5	22.6	13.9
Spill	ERMO.9	15.7	16.6	20.4	19.1	25.8	22.1	####	35.7	29.1	39.8	18.5	32.5	7.11	####	20.2	31.3	21.9	15.1	19.6	10.3
Spill	CRM1.5	12.9	18.3	18.4	20.7	23.6	19.8	####	29.9	30.8	39.4	21.8	21.3	14	####	20.8	13.1	29.1	12.7	21.7	12.3
LMBASS																					
Ref	CRM8.0	17.8	15	21.6	20.6	18.6	18.9	####	36.2	34.6	34.6	43.3	22.4	30.1	####	42	22.7	25.9	24.6	34.3	15.9
Ref	ERM8.0	18.9	21.1	20.8	19.3	21.3	21.1	####	30.9	36.6	23.8	26	29.9	18.4	####	34.3	17.4	35.4	20.5	47.9	17.9
Ref	LERM2.0		18.8	22.4	19.3	20.8	23.4	####		26.6	24.3	25.1	29.8	28.3	####		25.3	38.5	20.3	26	24.5
Spill	ERM3.0	18.4	18.4	24	20.3	21.7	21.5	####	29.6	36.1	28	32.3	30.6	21.8	####	25.3	18.5	42	17.9	44.4	17
Spill	ERMO.9	18.8	20	20.4	20.8	23.1	21.4	####	31.7	40.6	32.5	37.1	28.9	24	####	35	24.8	30.9	20.4	35.6	27.8
Spill	CRM1.5	18.9	16.4	22.8	20.9	23.4	21.8	####	31.1	25.9	31.3	36.3	34.1	24.1	####	32.1	43.3	35	21	27.1	13.9
AMY						BPRO						BUN									
	2009		2010		2011		2009		2010		2011		2009		2010		2011				
	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall			
BLUGIL																					
Ref	CRM8.0	43.7	21.6	58.5	31.6	63.2	24.8	####	41.2	34.9	41.6	38.8	41.2	37.9	####	3.58	2.67	2.63	1.88	2.1	1.33
Ref	ERM8.0	36.1	20.1	42.6	35.1	78	31.6	####	37.4	36.3	43	38.6	42	40.3	####	2.44	2.56	2.71	3	3.2	2.67
Ref	LERM2.0		21.4	56.2	30.6	65.6	29.1	####		35.6	41.1	37.6	43	40.6	####		2.2	2.7	2.43	1.78	2.56
Spill	ERM3.0	53.8	19.5	47.9	28.6	52.4	22	####	38.5	32.6	41.3	39.3	42	40.1	####	3.38	1.75	2.09	2.75	2.11	2.44
Spill	ERMO.9	52.7	18.1	65.1	24.3	64.6	28.3	####	40	39.6	41.1	38.6	46	42.6	####	3.56	2.14	2.25	1.38	2.13	3.11
Spill	CRM1.5	25.7	20.9	54.9	29.7	65.7	25.8	####	34.5	37.8	37.1	40.3	43.1	39	####	2	2.11	2.1	1.89	1.78	2
LMBASS																					
Ref	CRM8.0	159	152	220	224	207	186	####	52.2	44.7	59.4	48.9	43.9	45.2	####	3.2	2.86	3.75	2.25	2.43	2.44
Ref	ERM8.0	214	201	236	194	184	216	####	49	45.8	46.6	46.9	48.1	48.4	####	2.24	1.88	2.25	2.63	2.71	2.13
Ref	LERM2.0		205	185	198	209	262	####		44.5	50.4	45.1	51.3	45.8	####		2	1.88	2.88	3.78	2.63
Spill	ERM3.0	151	173	211	179	242	206	####	53.6	44.5	57.3	49.6	50.2	48.1	####	3.29	2.75	1.86	2.29	4.78	4.13
Spill	ERMO.9	193	227	234	284	298	220	####	51.9	53.5	60.6	47.9	52.6	47.9	####	3.36	3.38	3.38	2	4	3
Spill	CRM1.5	189	189	231	270	266	209	####	52	46.6	55.5	50.3	55	49.1	####	1.94	2.75	2.5	2.63	4.75	2.88
CA						CREAT						GLOB									
	2009		2010		2011		2009		2010		2011		2009		2010		2011				
	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall			
BLUGIL																					
Ref	CRM8.0	19.2	13	21.8	14.1	19.7	13.2	####	0.05	0.02	0.05	0.06	0.01	0.01	####	25	21.1	21.9	19.6	21.3	19.4
Ref	ERM8.0	15.3	13.1	20.2	14.7	18.7	14.2	####	0.12	0	0.03	0.03	0	0.06	####	22.8	20.4	20.7	19.6	20.2	19.1
Ref	LERM2.0		12.2	19.9	13.8	19.5	13.9	####		0.12	0.11	0.03	0.01	0	0 ####		18.9	21.4	17.9	19	16.1
Spill	ERM3.0	16.9	11.3	19.9	14.5	19.3	14.1	####	0.06	0.04	0.1	0	0	0.07	####	22.9	19.1	21.3	19.8	18.4	18.7
Spill	ERMO.9	18.9	13.8	18.8	14.1	20	15.3	####	0.08	0.07	0.08	0.09	0	0.08	####	24.3	23	20.6	19.8	20.1	20.7
Spill	CRM1.5	15.4	13.6	19.6	14.7	19.2	14	####	0.08	0.09	0.04	0.16	0.01	0.06	####	21.9	19.6	17.1	19.6	19.8	19.2
LMBASS																					
Ref	CRM8.0	18.4	12.4	19.3	14.4	15.3	13.2	####	0.02	0.11	0.28	0.18	0.1	0.09	####	34.6	30.3	37.9	28.4	25.4	26.2
Ref	ERM8.0	16.2	13.8	19.1	13.1	19.7	14.8	####	0.14	0.03	0.14	0.09	0.1	0.18	####	30.1	24.6	26	27.8	26.9	27.4
Ref	LERM2.0		13.2	20.2	13.1	17.6	13.9	####		0.09	0.16	0.05	0.16	0.03	0 ####		25.8	28	26	30.8	22.4
Spill	ERM3.0	19.7	13.3	20.3	14.2	18.2	14.4	####	0.06	0.05	0.11	0.03	0.09	0.16	####	35.1	26	33.1	29.4	28.3	26.5
Spill	ERMO.9	17.5	14.9	17	15.3	18.4	14.2	####	0.13	0.25	0.29	0.58	0.15	0.05	####	33	33.4	30.9	27.4	29.5	26.3
Spill	CRM1.5	17.3	13	21.9	14.3	19.2	13.5	####	0.13	0.08	0.15	0.18	0.16	0.18	####	33.9	25.9	33	29.1	31.5	27.8

Table 2. Fish health metrics for 7 blood parameters and 2 condition indices for bluegill and largemouth bass collected at 6 sites over three years. Parameters include GLUcose, HematoCriT, Potassium (K), LeuCocriT, Sodium (NA), PHOSphorus, Total BILirubin, Condition Factor, and Liver Somatic Index. Cells within each species group are color-coded with lowest values in light shades and highest values in dark shades.

		GLU					HCT					K										
		2009		2010		2011	2009		2010		2011	2009		2010		2011						
		Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring						
BLUGIL																						
Ref	CRM8.0	66.9	56	47.3	41.5	57.4	46.9	####	25.8	23.6	26.8	27.4	30.5	28.8	####	5.78	6.94	3.59	5.66	3.45	5.24	
Ref	ERM8.0	63.7	67.4	68.9	53.5	56	46	####	27.9	24.2	38.3	26.8	27.6	31.7	####	5.24	4.83	4.2	5.18	4.08	3.9	
Ref	LERM2.0	39.1	48.8	41.9	54	55.3	55.3	####	21.6	31.7	25	31.7	30.9	30.9	####	4.72	2.5	5.29	2.2	5.58		
Spill	ERM3.0	51.4	43	59.9	76.3	63.1	54.2	####	28.1	24.3	29.2	28	28.3	29	####	6.04	5.39	3.95	4.31	3.16	4.09	
Spill	ERMO.9	62.3	91.9	54.9	50.9	61.4	46.6	####	25.3	34.9	33.4	27.5	30.8	28.8	####	5.57	6.4	4.81	5.8	3.01	5.19	
Spill	CRM1.5	75.7	58.6	93.6	40.7	71.2	48.1	####	25.9	24.9	29.9	26.3	32.7	34.1	####	4.92	5.52	3.78	5.46	3.53	3.93	
LMBASS																						
Ref	CRM8.0	66.2	56.6	100	57	47	43.1	####	30.5	34.9	36.5	31	27.3	32.1	####	4.4	3.14	1.55	2.81	3.33	2.01	
Ref	ERM8.0	76.4	42.6	51.6	49.4	61.1	60.9	####	35	34	31	30	32.3	35	####	2.75	2.86	1.99	2.63	2.13	2.61	
Ref	LERM2.0	36	65.3	55	76.9	50.4	####		29.9	29.6	30.1	27.9	39.1	39.1	####	2.09	1.58	2.66	2.03	1.63		
Spill	ERM3.0	98.7	58.1	145	73.1	105	67.3	####	31	31.5	37.4	34	31	32.6	####	2.14	3.09	1.17	2.31	2.8	1.56	
Spill	ERMO.9	68.5	140	113	68	86	55	####	31.5	42.3	38.5	33.3	32.6	32.1	####	2.67	2.61	1.49	2.7	2.03	2.05	
Spill	CRM1.5	67.4	46.4	174	67.1	105	49	####	29.8	28.6	38.4	35.8	31.6	32.1	####	3.86	4.95	1.43	2.15	2.09	1.64	
LCT					NA					PHOS												
		2009	2010	2011		2009	2010	2011		2009	2010	2011		2009	2010	2011						
		Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	
BLUGIL																						
Ref	CRM8.0	0.81	1.06	1	1.13	0.75	1	####	152	151	148	153	155	151	####	12.6	9.16	13.5	7.89	13.1	8.52	
Ref	ERM8.0	0.5	0.61	0.57	1	1.2	0.94	####	151	147	151	156	148	148	####	11	8.73	11.9	10.5	12.3	8.84	
Ref	LERM2.0		0.8	0.6	1.07	1.11	1.06	####		149	147	151	150	152	152	####		6.39	11	8.19	11.1	9.74
Spill	ERM3.0	0.8	1.5	0.91	1	1.15	1.39	####	154	142	148	152	153	150	####	10	7.09	11.2	10.9	13.2	10.1	
Spill	ERMO.9	0.91	0.81	0.56	0.75	1.22	0.78	####	156	155	151	153	155	152	####	10.8	13.2	12.6	8.84	12.9	8.96	
Spill	CRM1.5	0.93	0.78	0.7	0.94	1.11	0.89	####	148	153	148	155	154	148	####	9.95	8.64	15	8.72	12.7	8.81	
LMBASS																						
Ref	CRM8.0	0.67	0.29	0.06	0.58	1	0.83	####	157	148	155	155	153	150	####	7.6	7.47	10.9	10.3	7.29	7.54	
Ref	ERM8.0	0.58	0.44	1.5	0.69	1.13	0.69	####	156	149	152	150	151	150	####	9.62	9.13	9.2	7.6	8.64	8.78	
Ref	LERM2.0		0.56	1.38	0.69	0.89	0.25	####		149	152	154	150	153	153	####		7.13	8.79	8.74	6.86	8.93
Spill	ERM3.0	1.29	0.63	0.75	0.5	0.78	0.63	####	156	152	155	155	154	151	####	9.81	8.54	12.3	9.09	7.03	9.01	
Spill	ERMO.9	1.18	0.56	0.56	0.5	0.81	0.81	####	157	160	153	156	152	150	####	9.17	12.7	8.49	8.55	8.79	9.99	
Spill	CRM1.5	0.86	0.64	0.29	0.69	0.75	1	####	152	152	155	155	155	147	####	9.05	9.96	16.5	9.69	6.73	7.58	
TBIL					CF					LSI												
		2009	2010	2011		2009	2010	2011		2009	2010	2011		2009	2010	2011						
		Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	
BLUGIL																						
Ref	CRM8.0	0.39	0.51	0.34	0.54	0.67	0.62	####	1.81	1.66	1.85	1.74	1.66	1.70		1.40	0.72	1.93	0.77	1.91	0.80	
Ref	ERM8.0	0.53	0.41	0.54	0.89	0.46	0.96	####	1.76	1.63	1.67	1.69	1.73	1.71		1.80	1.10	2.03	1.00	1.85	0.83	
Ref	LERM2.0		0.7	0.37	0.73	0.5	0.96	####		1.66	1.7	1.75	1.73	1.55			0.98	1.90	0.87	1.83	0.77	
Spill	ERM2.0	0.43	0.39	0.3	0.74	0.38	0.8	####	1.68	1.56	1.84	1.77	1.71	1.74		1.30	0.73	1.89	0.92	1.61	0.73	
Spill	ERMO.9	0.34	0.61	0.33	0.43	0.51	0.54	####	1.73	1.66	1.82	1.69	1.66	1.71		1.30	0.82	1.54	0.66	1.50	0.76	
Spill	CRM1.5	0.45	0.46	0.45	0.61	0.54	0.46	####	1.64	1.66	1.72	1.78	1.66	1.72		1.20	0.79	1.61	1.62	1.50	0.80	
LMBASS																						
Ref	CRM8.0	0.22	0.39	0.3	0.4	0.21	0.36	####	1.34	1.21	1.57	1.32	1.40	1.34		1.49	0.84	1.76	0.64	1.46	0.60	
Ref	ERM8.0	0.31	0.46	0.28	0.44	0.26	0.44	####	1.43	1.38	1.37	1.31	1.40	1.31		1.47	0.73	1.14	0.74	1.47	0.84	
Ref	LERM2.0		0.48	0.24	0.46	0.24	0.43	####		1.37	1.41	1.35	1.37	1.22			1.07	1.26	0.70	1.47	0.67	
Spill	ERM2.0	0.26	0.36	0.26	0.5	0.21	0.46	####	1.42	1.30	1.54	1.39	1.70	1.28		1.84	0.82	1.32	0.94	1.44	0.71	
Spill	ERMO.9	0.22	0.45	0.3	0.51	0.26	0.44	####	1.50	1.29	1.44	1.38	1.57	1.26		1.70	0.60	1.40	0.72	1.33	0.59	
Spill	CRM1.5	0.35	0.3	0.25	0.39	0.23	0.46	####	1.42	1.37	1.43	1.41	1.56	1.30		1.75	0.75	1.54	0.76	1.55	0.61	